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Treatment Outcomes and Tumor Loss of Heterozygosity in Germline DNA Repair–deficient Prostate Cancer

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Abstract

Background: Germline mutations in DNA repair genes were recently reported in 8–12% of patients with metastatic castration-resistant prostate cancer (mCRPC). It is unknown whether these mutations associate with differential response to androgen receptor (AR)-directed therapy.
Objective: To determine the clinical response of mCRPC patients with germline DNA repair defects to AR-directed therapies and to establish whether biallelic DNA repair gene loss is detectable in matched circulating tumor DNA (ctDNA).
Design, setting, and participants: We recruited 319 mCRPC patients and performed targeted germline sequencing of 22 DNA repair genes. In patients with deleterious germline mutations, plasma cell-free DNA was also sequenced.
Outcome measurements and statistical analysis: Prostate-specific antigen response and progression were assessed in relation to initial androgen deprivation therapy (ADT) and subsequent therapy for mCRPC using Kaplan–Meier analysis.
Results and limitations: Of the 319 patients, 24 (7.5%) had deleterious germline mutations, with *BRCA2* (n = 16) being the most frequent. Patients (n = 22) with mutations in genes linked to homologous recombination were heterogeneous at initial presentation but, after starting ADT, progressed to mCRPC with a median time of 11.8 mo (95% confidence interval [CI] 5.1–18.4). The median time to prostate-specific antigen progression on first-line AR-targeted therapy in the mCRPC setting was 3.3 mo (95% CI 2.7-3.9). Ten out of 11 evaluable patients with germline *BRCA2* mutations had somatic deletion of the intact allele in ctDNA. A limitation of this study is absence of a formal control cohort for comparison of clinical outcomes.
Conclusions: Patients with mCRPC who have germline DNA repair defects exhibit attenuated responses to AR-targeted therapy. Biallelic gene loss was robustly detected in ctDNA, suggesting that this patient subset could be prioritized for therapies exploiting defective DNA repair using a liquid biopsy.
Patient summary: Patients with metastatic prostate cancer and germline DNA repair defects exhibit a poor response to standard hormonal therapies, but may be prioritized for potentially more effective therapies using a blood test.

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1. Introduction

Prostate cancer is one of the most heritable human cancers [1,2]. Two recent studies reported that germline mutations in DNA damage repair genes, including *BRCA2*, are present in 8–12% of patients with metastatic prostate cancer [3,4]. This frequency is significantly higher than in localized prostate cancer or within the general population, suggesting a strong association with aggressive disease [4–6]. Indeed, in localized prostate cancer, *BRCA2* germline mutations are associated with early-onset, high-grade, and poor-prognosis disease [6,7]. However, the clinical impact of germline DNA repair mutations in metastatic prostate cancer is less clear.

Although metastatic prostate cancer is initially treated with androgen deprivation therapy (ADT), it inevitably progresses to metastatic castration-resistant prostate cancer (mCRPC). In this setting, additional targeting of the androgen receptor (AR) signaling axis with abiraterone or enzalutamide is efficacious in the majority of patients, but duration of response is highly variable [8,9]. Biomarkers to predict poor therapy response are beginning to emerge, particularly modifications to the AR gene and transcript [10–12]. The degree to which germline and somatic DNA repair mutations influence patient response to ADT or AR-targeted therapy is unknown. Since DNA damage repair deficiency can result in high genomic instability in cancer [13], there may be an increased potential for evolution of treatment-resistant clones. Conversely, the presence of DNA repair gene defects was recently associated with the response of mCRPC to the poly ADP ribose polymerase (PARP) inhibitor olaparib and platinum-based chemotherapies [14–16]. Practical tumor biomarkers will be important for optimal clinical use of PARP inhibitors. Biallelic DNA repair gene loss is likely to be the strongest predictor of response [17], meaning that in patients with a germline mutation, detection of a second somatic "hit" is important. While tissue biopsies may not be feasible outside of large cancer centers, surveying of circulating tumor DNA (ctDNA) for somatic DNA repair defects appears promising [11].

In this study, we aimed to validate the prevalence of germline DNA repair defects in a cohort of mCRPC patients, to determine the clinical response of this patient subset to AR-directed therapy and to establish whether biallelic DNA repair gene loss is detectable in matched ctDNA.

2. Patients and methods

2.1. Patient cohort

We recruited 319 consecutive mCRPC patients enrolled in our liquid biopsy program at the Vancouver Prostate Centre and British Columbia Cancer Agency (BCCA) from August 2013 to August 2016. This included 161 patients from an ongoing multicenter randomized phase II crossover trial of enzalutamide versus abiraterone (NCT02125357), 30 patients with poor-prognosis mCRPC enrolled on an ongoing randomized phase II trial of cabazitaxel versus abiraterone or enzalutamide (NCT02254785), 24 mCRPC patients from the SU2C/PCF/AACR West Coast Prostate Cancer Dream Team study [18], and 104 patients with mCRPC receiving standard of care therapy at the BCCA Vancouver Centre. Importantly, patients were unselected for age at diagnosis, second primary cancer, or a familial cancer history. Where available, we retrieved clinical data from electronic medical records. For localized disease, risk assessment at diagnosis was determined using the University of California, San Francisco – Cancer of the Prostate Risk Assessment (UCSF-CAPRA) score [19]. Castration resistance was defined according to American Urological Association (AUA) guidelines [20], and progression-free survival (PFS) on AR-targeted therapies was defined as the number of days from therapy initiation to a rise in serum prostate-specific antigen (PSA) of 25% or greater and an absolute increase of 2 ng/ml or more from the documented nadir [21]. PFS was assessed using Kaplan–Meier analysis and the log rank test. Approval for this study was granted by the University of British Columbia Ethics Board or the local ethics review board. Written informed consent was obtained from all participants prior to enrollment.

2.2. Sample collection and processing

For the first 93 mCRPC patients recruited, germline DNA was extracted from whole blood (collected in ethylenediaminetetraacetic acid [EDTA] tubes) using a standard phenol–chloroform protocol. For the remaining 226 patients, the buffy coat fraction was isolated from whole blood collected in either EDTA or Streck Cell-Free DNA BCT tubes, and germline DNA was extracted from buffy coat using the QIAGEN DNeasy Blood and Tissue kit. From a subset of patients with germline DNA damage repair defects, matched cell-free DNA (cfDNA) was extracted from up to 6 ml plasma with the QIAGEN Circulating Nucleic Acids kit, as previously described [11]. Quantification of cfDNA was performed using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, California, United States) and the Qubit dsDNA HS Assay Kit, as well as a NanoDrop 2000 spectrophotometer.

2.3. Targeted DNA sequencing

We employed a targeted sequencing strategy using a custom NimbleGenSeqCap EZ Choice Library and Illumina machines. Our standard prostate cancer design (used in house for all mCRPC tumor studies) captures the exonic regions of 73 genes, including the following 22 DNA damage repair genes: *ATM*, *ATR*, *BRCA1*, *BRCA2*, *CDK12*, *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *FANCA*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *MLH1*, *MSH2*, *MSH6*, *PALB2*, *RAD51B*, and *RAD51C* (Supplementary Fig. 1). For each specimen, 10–100 ng of DNA was sheared into 180 bp fragments with a Covaris focused ultrasonicator, upon which A-tailing, end repair, adapter ligation, and subsequent polymerase chain reaction (PCR) amplification (12–17 cycles) was performed. Library quantification was carried out with the NanoDrop 2000 spectrophotometer, and each library was run on an ethidium bromide gel to confirm success. Sets of up to 25 purified libraries were multiplexed to obtain single pools with combined mass of 1 μg and hybridized to the capture panel for 16–20 h at 47 °C. The subsequent wash, recovery, and amplification of the captured regions were performed according to the NimbleGenSeqCap EZ system protocols. Final libraries were purified with Agencourt AMPure beads and quantitated with the KAPA qPCR kit. Pools were diluted to 20 pM and sequenced on the Illumina MiSeq (v3 600 cycle kit) or HiSeq 2500 (Rapid SBS Kit v2) platforms. For cfDNA sequencing, the same protocol was followed, except that shearing was not performed.

2.4. Bioinformatics

Reads were aligned against the hg38 reference genome using Bowtie-2.2.4 [22]. Optical and PCR duplicates were removed using samblaster-0.1.20 [23]. Per-base read coverages in target regions were counted using bedtools-2.25.0 [24]. Germline variants were called in white blood cell samples when supported by an alternate allele fraction of at least 15% and at least five reads. Additionally, the average allele fraction of the variant was required to be 20 times higher than the background error rate

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